Measurement of Fipronil and a metabolite in dosed rodents and human biological samples

R.L. McMahen[1,2], M. J. Strynar[2], D. Herr[3], G. Moser[3], S. Dagnino[1,2], S. Garantziotis[5], E. M. Andersen[2], L. McMillan[4,2], A. B. Lindstrom[2]

1. ORISE Fellow, U.S. Environmental Protection Agency, National Exposure Research Laboratory, Research Triangle Park, North Carolina 27711, USA.
2. U.S. Environmental Protection Agency, National Exposure Research Laboratory, Research Triangle Park, North Carolina 27711, USA.
3. U.S. Environmental Protection Agency, National Health Effects Research Laboratory, Research Triangle Park, North Carolina 27711, USA.
4. SEEP Employee, , U.S. Environmental Protection Agency, National Exposure Research Laboratory, Research Triangle Park, North Carolina 27711, USA.
5. National Institute for Environmental and Health Sciences, Research Triangle Park, North Carolina 27711, USA.

Introduction:

- Fipronil (shown below) is a phenyl pyrazole insecticide that is used to control ants, fleas, termites, roaches, and other pests[1]
- Fipronil is used in residential and agricultural settings and on golf courses
- Its widespread use leads to contamination of indoor and outdoor dust[2] and water sources[3]
- This contamination leads to the high potential for human exposure
- In order to measure human exposure, a good biomarker first has to be identified

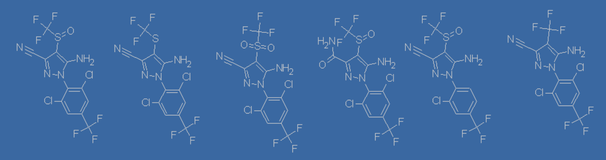
Examples of products containing fipronil:

Frontline- flea protection

Regent- broad spectrum insecticide

Goliath gel- cockroaches

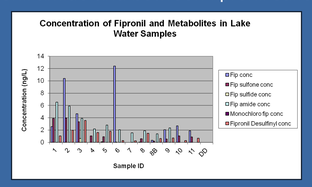
Fipronil and derivatives:

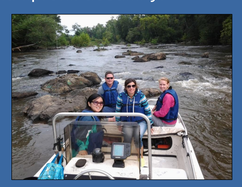


Fipronil | Fipronil sulfide | Fipronil sulfone | Fipronil amide | Monochloro fipronil | Fipronil desulfinyl

Fipronil in a local water body:

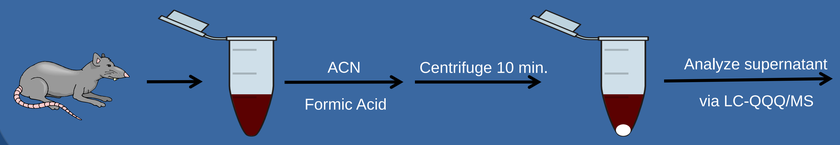
- Water samples were analyzed for fipronil, fipronil sulfone, fipronil sulfide, fipronil amide, monochloro fipronil, and fipronil desulfinyl photodegradate[4]
- Most fipronil metabolites were found in low levels
- Statistics show a significant positive correlation between fipronil sulfone and fipronil amide and between fipronil sulfone and fipronil desulfinyl





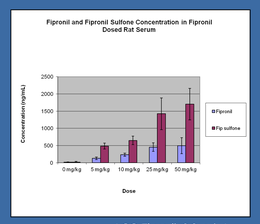
Identifying a biomarker of exposure:

- Adult male Long Evans rats were dosed with Fipronil in 2 separate studies-
  1. Acute doses of either 5 ,10, 25 or 50 mg/kg body weight and sacked at 6hr.
  2. Repeated doses of either 5 or 10 mg/kg every 24 hours for 2 weeks and sacked 6 hr. after the last dose
- The serum was collected
- 25 uL of serum was extracted via a formic acid denature and an ACN protein crash
- The serum sample was centrifuged and the supernatant was analyzed via LC-TOF/MS (for discovery) and LC-QQQ/MS (for quantitation)

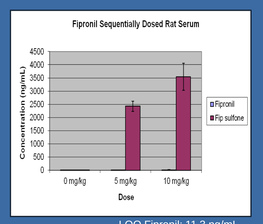


Results of Dosed Rat Study:

- Serum samples were analyzed for fipronil and metabolites via LC-TOF/MS
- Small amounts of fipronil were found in sequentially dosed and acutely dosed rat serum samples
- Fipronil sulfone was the primary metabolite and was identified as a biomarker of exposure to fipronil
- None of the other metabolites contained in the LC-QQQ/MS method were found in the serum samples
- Graphs of the quantitation results are shown below for both the acutely dosed and sequentially dosed rat serum samples



LOQ Fipronil: 6.2 ng/mL
LOQ Fip sulfone: 4.5 ng/mL



LOQ Fipronil: 11.3 ng/mL
LOQ Fip sulfone: 8.6 ng/mL

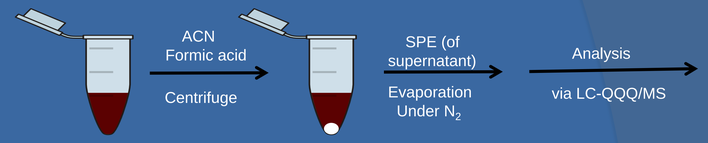
Instrumentation:

LC system: Agilent 1100
QQQ mass spec system: Sciex 3000

LC system: Agilent 1100
TOF mass spec system: Agilent 6200

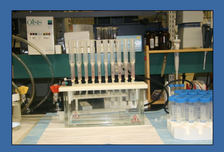
Biomarkers of exposure to fipronil in human serum samples:

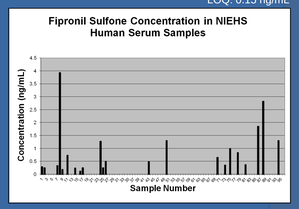
- 200 uL of human serum obtained from NIEHS was extracted via a formic acid denature, an ACN protein crash
- After centrifugation the supernatant was extracted onto an HLB SPE cartridge and eluted with ACN
- Solvent was removed from the eluate, and the solution was analyzed via LC-QQQ/MS



Results of human exposure study:

- Serum samples were analyzed for fipronil, fipronil sulfone, fipronil sulfide, fipronil amide, and monochloro fipronil
- Fipronil sulfone was the only metabolite found in the human serum
- Fipronil sulfone was found in approximately 23% of the samples
- Mean: .20 +/- .58 ng/mL
- Range: 0-3.93 ng/mL





Conclusions:

- The primary biomarker of exposure to fipronil was identified as fipronil sulfone
- Fipronil and derivatives were found in a local water body
- Fipronil sulfone (the biomarker of exposure to fipronil) was found in low levels in human serum

Our lab:

- Andy Lindstrom, PhD
- Mark Strynar, PhD
- Sonia Dagnino, PhD
- Erik Andersen
- Larry McMillan
- Shuang Liang

References: 1. Colliot, F., Kukorowski, K. A., Hawkins, D. W., and Roberts, D. A. (1992) Fipronil: a new soil and foliar broad spectrum insecticide. In Brighton Crop Protection Conference-Pests and Diseases, Vol. 1, pp 29-34, British Crop Protection Council, Farnham, U.K. 2. *Environ. Sci. Technol.*, **2009**, *43* (15), pp 5665–5670 3. *Environ. Sci. Technol.*, **2012**, *46* (3), pp 1489–1495 4. Colin, C.D., et al. (2003) Fipronil: Environmental Fate, Ecotoxicology, and Human Health Concerns. *Rev. Environ. Contam. Tox.*, **2003**, *176*, 1-66.